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Nanomechanics of electrospun phospholipid fiber

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Electrospun asolectin phospholipid fibers were prepared using isooctane as a solvent and had an average diameter of 6.1 ± 2.7 μm. Their mechanical properties were evaluated by nanoindentation using Atomic Force Microscopy, and their elastic modulus was found to be approximately 17.2 ± 1 MPa. At a cycle of piezo expansion-retraction (loading-unloading) of a silicon tip on a fiber, relatively high adhesion was observed during unloading. It is proposed that this was primarily due to molecular rearrangements at the utmost layers of the fiber caused by the indentation of the hydrophilic tip. The phospholipid fibers were shown to be stable in ambient conditions, preserving the modulus of elasticity up to 24 h. © *2015 AIP Publishing LLC*.
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Phospholipids have received particular attention in the fabrication of advanced biomaterials due to their ability to self-assemble in highly organized structures, similar to cell membranes, with a specific permeability to ions and molecules.[1,2] Phospholipids have therefore been used to prepare biomimetic capsular structures (mainly liposomes or micelles),[3–6] films, coatings for water microdroplets,[2] and microbubbles[7] with a broad range of applications, including drug delivery,[8] cell encapsulation,[9] and tissue engineering.[2]

McKee and co-authors[10,11] have shown that a solution of phospholipids above 35% w/w in DMF:CHCl3 [3:2 v/v] is capable of forming continuous fibers using electrospinning processing with an average phospholipid fiber diameter of ~3.3 μm (for 45% w/w solution). In the present study, we report that isooctane is also an efficient solvent for the electrospinning of asolectin fibers at a phospholipid concentration of 60% w/w. Moreover, nanoindentation using Atomic Force Microscopy (AFM) has been used to study the mechanical properties of electrospun fibers.[12] For instance, AFM has been used to explore nanomechanical properties of electrospun fibers made of synthetic (e.g., poly-L-lactic acid (PLLA),[12] polyvinyl alcohol (PVA),[13] and polyacrylonitrile (PAN)[14] and natural macromolecules (gelatin, collagen, and elastin[15]). Nanomechanical studies of nano-microfibers comprising phospholipids have focused on lipid bilayers[16] and phospholipid-based structures such as phospholipid microbubbles.[7] The mechanical properties of electrospun polymerizable lipid-based materials, namely, cholesteryl-succinyl silane (CSS) nanofibers were also investigated.[17] However, no studies have been carried out to investigate the nanomechanical properties of unmodified phospholipid nanofibers. This study reports the formation of electrospun asolectin fibers using isooctane and the evaluation of their nanomechanical properties.

Asolectin from soybean (Sigma Aldrich product no.: 11145, lot no.: BCB66221V) was used, containing roughly 25%–33% of lecithin, cephalin, and phosphatidylinositol, 24% saturated fatty acids, 14% mono-unsaturated, and 62% polyunsaturated fatty acids. The asolectin was dissolved in isooctane at a concentration of 60% w/w and was allowed to stir overnight at room temperature. A high voltage power supply (ES50P-10W, Gamma High Voltage Research, Inc., USA) was used to provide a voltage of 20 kV through the needle to the phospholipid solution. A syringe pump (New Era Pump Systems, Inc., USA) was used to feed the asolectin solution at a feed rate of 0.01 ml/min. The electrospinning process was carried out at ambient conditions. Phospholipid fibers were collected on a grounded steel plate covered with aluminum foil placed at a distance of 12 cm from the end of the needle. The main reason for the formation of electrospun phospholipid fibers is that, with the increase of the phospholipid concentration, a transition occurs from single molecules to micelles, then to the formation of rod-like structures, and finally a creation of elongated aggregates that can be electrospun.[10] Isooctane is thus a suitable solvent to electrospun phospholipids, since it is known to promote the formation of elongated cylindrical reverse micelles[18] and supramolecular structures of lecithin solutions.

For AFM and optical microscopy studies, a piece of silicon wafer and a microscope glass slide were placed on top of a metallic collector, respectively, to further allow the collection of fibers on these substrates. The collected fibers were dried overnight at room temperature. Morphology was assessed by scanning electron microscopy (SEM) and optical microscopy. For SEM, the specimens were mounted on aluminum stubs and sputter-coated with gold prior to the visualization on a scanning electron microscope (FEI Inspect S). The cross-sections of the microfibers were analyzed, applying a cryogenic technique to fracture the samples, before coating and imaging in a Quanta FEG 3D SEM. The diameters of the electrospun fibers were measured by using an image visualization software Image-J (National Institutes of Health, USA). The average fiber diameters and diameter distributions were determined by measurements of 100 individual fibers. As shown in Figure 1, uniform and very smooth fibers without beads were achieved via electrospinning of 60% w/w asolectin solution in isooctane. Figures 1(c) and 1(d) show that asolectin electrospun fibers are uniform over

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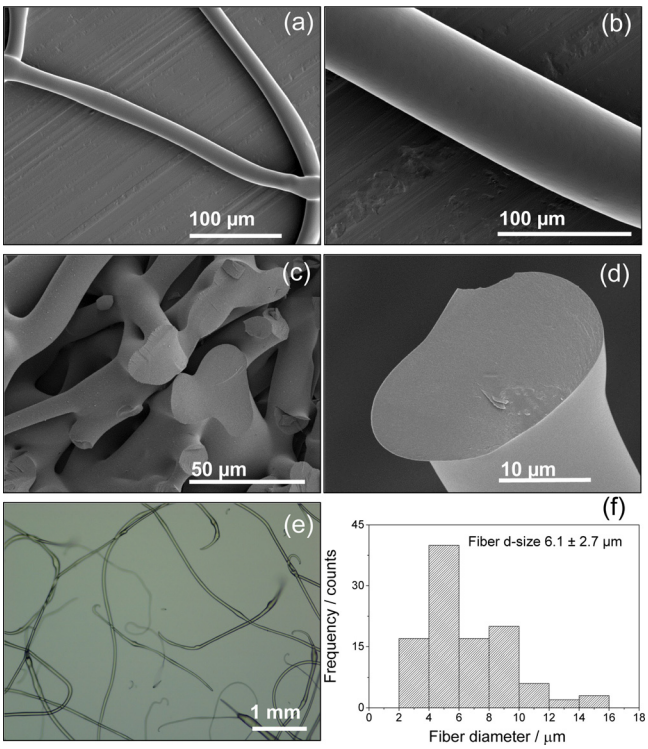

FIG. 1. SEM images of (a) electrospun asolectin microfibers, (b) a single fiber at a higher magnification showing the smooth surface of the fiber, (c) cross-section of several and (d) single fiber at higher magnification. (e) Image of asolectin fibers by optical microscopy and (f) distribution of fiber diameters.

its cross-section. Resultant fibers had an average diameter of $6.1 \pm 2.7\ \mu$m.

Nanomechanics experiments were conducted using AFM with a Dimension Edge AFM (Bruker, Santa Barbara, CA). Force-distance measurements were carried out in ambient conditions at a tip speed of $0.50\ \mu$m/s. Rectangular silicon cantilevers (model FMV, cone angle 21°, Bruker) were employed with nominal spring constants of 2.8 N/m. The normal spring constant of the cantilevers was obtained with the generalized Sader method.[19,20] The normal photodetector sensitivity (nm/V) was acquired from the slope of the linear part of force curves at the repulsive regime, obtained on the flat regions of a silica specimen. All bare probes were cleaned using piranha solution prior to use. The probes were subsequently rinsed with copious amounts of ultrapure water, dried in a flow of nitrogen, and used immediately.

Nanomechanical analysis of the force-distance curves was carried out according to Sirghi and Rossi[21] In short, the contact part of the force versus indentation curve during piezo retraction (unloading) was fitted according to the following equation:

$$F(\delta) = -\frac{4\gamma_a r_a}{\cos a} + \left(2E^* r_a - \frac{8\gamma_a \tan a}{\pi \cos a}\right)\delta + \frac{2E^* \tan a}{\pi}\delta^2, \tag{1}$$

where F is the applied load, γ_a is the thermodynamic work of adhesion, r_a is the contact radius at zero indentation depth, α is the cone angle of the tip, δ is the indentation depth, and E^* is the reduced modulus of the materials in contact. Since the Young's modulus of the silicon tip, E_t, is several orders of magnitude higher than that of the fiber sample, the reduced modulus can be approximated to $E^* \approx E_s/(1-\nu_s^2)$, where ν_s is the Poisson ratio of the fiber sample and E_s its Young's modulus.[21–23] For the cone angle, α, the manufacturer's values were used (21°), while ν_s was assumed to be 0.3.[24,25]

Figure 2(a) shows a typical F-δ curve of a loading-unloading cycle of an FMV silicon tip on a fiber. In all measurements, the maximum indentation depth was maintained below 500 nm (<10% of the fiber's diameter) to avoid contributions from the SiO_2 substrate to the mechanical response of the fiber.[26] The fit of Eq. (1) in the unloading part of the curve (red line in Figure 2) is from the maximum applied load until the minimum one (F_{Adh}). In total, five fibers were characterized on at least four different places along their long axis direction, where 25 force-distance curves were obtained on each place. To ensure that the slope of the curved fiber surfaces did not affect the experiments, all measurements were made at the top of the fibers, at the center of their short axis. The results of the nanomechanical analysis are summarized in the inset of Figure 2. As a comparison, F-δ curves obtained from poly(dimethylsiloxane) (PDMS) surface are shown in Figure 2(b).

A most striking feature in Figure 2(a) is the high adhesion observed during unloading (black line) and adhesion hysteresis. While adhesion hysteresis is a generally observed phenomenon in loading/unloading cycles by AFM probes on soft matter, Figure 2(a) is particularly noticeable as the loading process is monotonically repulsive (blue line), a "snap-in" event is absent, and the hysteresis is enormous. In contrast, the experiment conducted with the same probe on PDMS substrate (Figure 2(b)) revealed smaller elasticity modulus E_s (ca. 1.52 MPa), i.e., soft mechanical properties, yet much smaller surface adhesion energy γ_a (ca. 0.23 Nm) and ignorable adhesion hysteresis. A similarly large hysteresis in the adhesion between loading/unloading of AFM probe

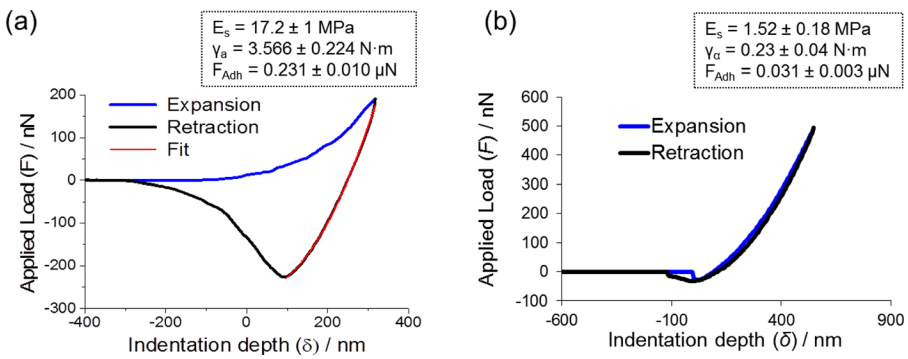

FIG. 2. A plot of the Applied Load (F) vs Indentation Depth (δ) for a cycle of piezo expansion-retraction (loading-unloading) of a silicon tip on an asolectin fiber (a). Inset: Average values of E_s, γ_a, and adhesion force F_{Adh}, as obtained from a total of more than 100 force-distance measurements on random locations across five asolectin fibers and (b) on a PDMS surface.

was reported in a previous study of CSS nanofibers,[17] where the core of the fiber is composed of hydrophilic silicate network, whereas the fiber surface is modified with hydrophobic steroid tails. Thus, a strong adhesion hysteresis was attributed to the indentation-induced contact between AFM probe and the hydrophilic core.[17] We propose that a similar "core-surface amphiphilicity" and the association mechanism of AFM probes with the hydrophilic inner side of the phospholipid fibers, such as hydrogen bonding formation, further contributes to high adhesion hysteresis, as shown in Figure 2(a). The lack of hysteresis for nano-indentation of PDMS (Figure 2(b)) can be explained by the lack of amphiphilicity for PDMS. In contrast to structural stability in isooctane or ambient, the fibers rapidly dissembled in aqueous environment, which further supports this model. Despite the similar nanomechanical responses, "core-surface amphiphilicity" of phospholipid fibers is derived from the intrinsic nature of phospholipids without the hydrophobization step needed for CSS nanofibers.[17]

The fibers were collected on the semi-conductive silicon wafer for the nanomechanical testing, and some of them show non-uniform diameters along their long axis. In particular, they possess some "knots" with slightly larger diameters at relatively regular intervals. The fiber illustrated in Figure 3 also possesses a "head" of considerably larger width than the rest of the fiber. As can be seen in Figure 3, the modulus values on the thin parts of the fibers (location "C" in Figure 3, for instance) are higher than those on the knot (location "D") and the "head" (location "A") by about 50%. Nevertheless, the differences in fiber diameter alone cannot explain the differences in the mechanical properties shown above. The diameter at location C is only 20% lower than that at location D, yet the modulus value is about 45% higher at location C. On the other hand, the modulus at location A is statistically equal to that at location D, while the difference in their diameter (or width) is about 285%.

The average elastic modulus of asolectin fiber was about 17.2 ± 1 MPa, resembling enhanced elastic properties with respect to the elastic modulus determined for other phospholipid based structures. For instance, the elastic moduli of natural cholesterol and phosphatidylcholine were found to be 0.27 MPa and 0.03 MPa, respectively.[27] Moreover, the elastic moduli of electrospun CSS nanofibers were found to be dependent on the hydrolysis and polymerization degree of CSS molecules.[17] The less polymerized fibers displayed an elastic modulus ranging from 0.8 to 1 MPa, while the elastic modulus of the CSS fibers was determined to be 55.3 ± 27.6 to 70.8 ± 35 MPa.[17] Furthermore, the nanomechanical properties of phospholipid microbubbles were also investigated by AFM using distinct mechanical models.[7] Depending on the methodology used, the Young's modulus is reported to range from approximately 8 to 38 MPa (Ref. 7) and to be 133 kPa when determined following the Hertz theory on an equivalent homogeneous sphere.[7]

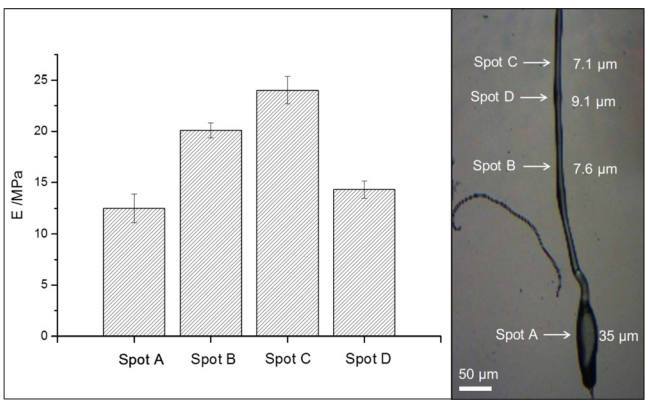

FIG. 3. Variation of elastic modulus (E_s) with fiber diameter at the different locations examined in the same fiber and corresponding locations examined by bright field microscopy.

For comparison, the elastic modulus determined for electrospun nanofibers made of synthetic polymers, such as PLLA, PVA, and PAN, have been found to range from 0.5 to 0.9 GPa,[12] 4 to 13 GPa,[13] and 5.72 to 26.55 GPa,[14] respectively, depending on the size of the diameter of the fiber. In addition, electrospun nanofibers made of natural materials, such as proteins like gelatin, collagen, and elastin, exhibited a lower elastic modulus (tensile moduli), compared to synthetic polymers of approximately 426, 262, and 184 MPa, respectively.[15] Previous studies concerning the determination of Young's modulus of single electrospun polymeric fibers have found that the Young's modulus of fibers is regulated by the diameter of the fibers.[12,14,28,29] The mechanical behaviour of electrospun fibers was found to be a consequence of the changes in orientation of the polymer molecules during the electrospinning process provided by the strong strain forces of the polymer jets.[29] The high strain rate of the ejected polymer jets induces a molecular orientation of polymer nanofibers along the fiber axis.[29] However, in this respect, further studies are needed to investigate the behaviour of the molecular orientation of the phospholipids when they are submitted to electrospinning process and its effect on their mechanical properties. It should be noted that self-assembly of phospholipids into organized structures is known to be mediated by several factors, including the application of an external electrical field.[30]

The modulus of the fibers was also examined with respect to their stability in ambient conditions. The humidity of the ambient conditions was approximately 60%. As can be seen in Figure 4(a), exposure of the fibers to the humid environment up to 80 min did not affect their nanomechanical properties. In Figure 4(b), another location on the same fiber was also examined, and the modulus remained the same up to 20 min. The fiber was subsequently stored in a desiccator and the same location was examined after 24 h (red circles). The values obtained are statistically indistinguishable from those at 0 h.

In summary, the mechanical properties and stability of a single electrospun asolectin phospholipid fiber were investigated through the use of AFM. The elastic modulus of asolectin microfibers with an average diameter of 6.1 ± 2.7 μm prepared using isooctane was found to be approximately 17.2 ± 1 MPa.

The adhesion during loading was absent, which implies that the fiber surface is primarily hydrophobic. However, during unloading, a strong adhesion and consequently a strong adhesion hysteresis were observed. While adhesion hysteresis is a general behavior in indentation on soft matter

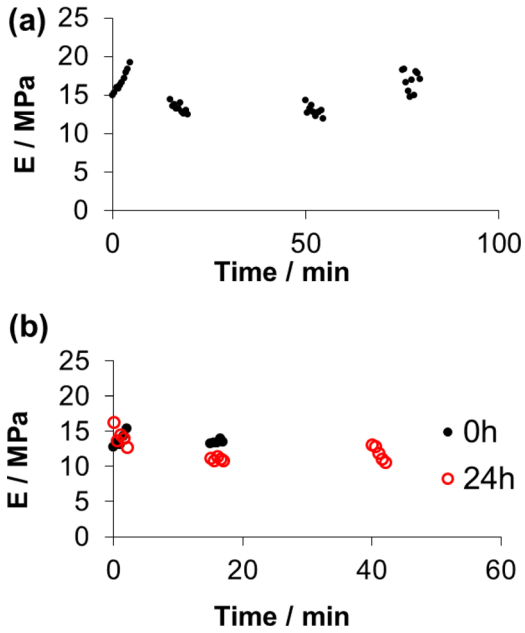

FIG. 4. (a) Values of E_s at the same location on a fiber as a function of time, obtained under ambient conditions. (b) Values of E_s at the same location on a fiber as a function of time, obtained under ambient conditions at 0 h (black dots) and after 24 h of storage in a desiccator (red circles).

by AFM, we propose that strong interaction between AFM probe, such as silanol groups on the tip, and buried inner parts of the fibers, such as polar heads of phospholipids, in ambient also contributed to the adhesion hysteresis.[17] Furthermore, the elastic modulus of phospholipid fibers was found to remain constant at ambient condition even after 24 h, supporting that the fibers remain stable under such conditions. Further, studies are needed to investigate the effect of solvents and electrospinning processing on the molecular orientation, morphology, and mechanical properties of asolectin phospholipid fiber.

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